How DNA Unwinds At the Onset of Transcription

August 20, 2012

EM Scherer

The process of synthesizing a protein from a specific DNA sequence, or gene, involves many steps at the molecular and cellular levels. In all cases, the respective DNA sequence must first be transcribed into an RNA sequence, which designates the order of amino acids that specify a protein's primary structure. RNA polymerase II (Pol II), the enzyme responsible for synthesizing RNA for protein translation, requires six general transcription factors to initiate this process. In addition, the double-stranded (ds)-DNA must be unwound to provide Pol II access to a 'template' strand that guides the synthesis of RNA. While the structure of the 'open complex' that facilitates RNA synthesis was previously established, it has remained unclear how the ds-DNA structure present in the Pol II pre-initiation complex is opened, and how the template strand is then inserted into Pol II's enzyme site to attain its position in the open complex. Although previous studies found that the transcription factors TFIIE and TFIIH are required for this transition, recent work by postdoctoral fellow Dr. Sebastian Grünberg and colleagues in the Hahn Lab of the Basic Sciences Division explains how they may be involved.

Using yeast transcription factor variants labeled with chemical probes to indicate the proximity of neighboring molecules, the authors found that the Tfa1 and Tfa2 subunits of TFIIE heterodimerize through their winged helix motifs, forming a unique triple winged helix fold. When incorporated into a working structural model of the pre-initiation complex, this fold spans the Pol II active site cleft, thereby encircling a region of ds-DNA that is upstream of the transcription start site.

Chemical probes placed at the dimerization interface also revealed interactions between TFIIE and TFIIH subunit Ssl2, an ATPase related in structure to DNA helicases. The model that best fits these data includes a form of Ssl2 that is ATP-bound, which in the pre-initiation complex orients the Ssl2 ATP-binding site toward TFIIE and places the predicted Ssl2 DNA-binding surface where it could bind DNA in the region of the transcription start site. In contrast, in the open complex model, this region of DNA is bound within the Pol II cleft, and Ssl2 most likely associates with downstream DNA.

In comparing models for these two 'before-and-after' complexes, Grünberg and colleagues proposed that Ssl2 in the pre-initiation complex translocates the template strand (and transcription start site) into the Pol II cleft by a right-handed threading mechanism, contrary to just rotating the DNA as suggested in an older model proposed a decade ago. In the new model, threading of the DNA

displaces the non-template strand onto the DNA-binding domain(s) of TFIIE and generates an open complex.

These results provide a better understanding of the interactions between components of the pre-initiation complex and have produced a testable model with which to investigate the molecular steps preceding transcription.

Grünberg S, Warfield L, Hahn S. 2012. Architecture of the RNA polymerase II preinitiation complex and mechanism of ATP-dependent promoter opening. *Nature Structural & Molecular Biology*, Epub ahead of print, doi: 10.1038/nsmb.2334.

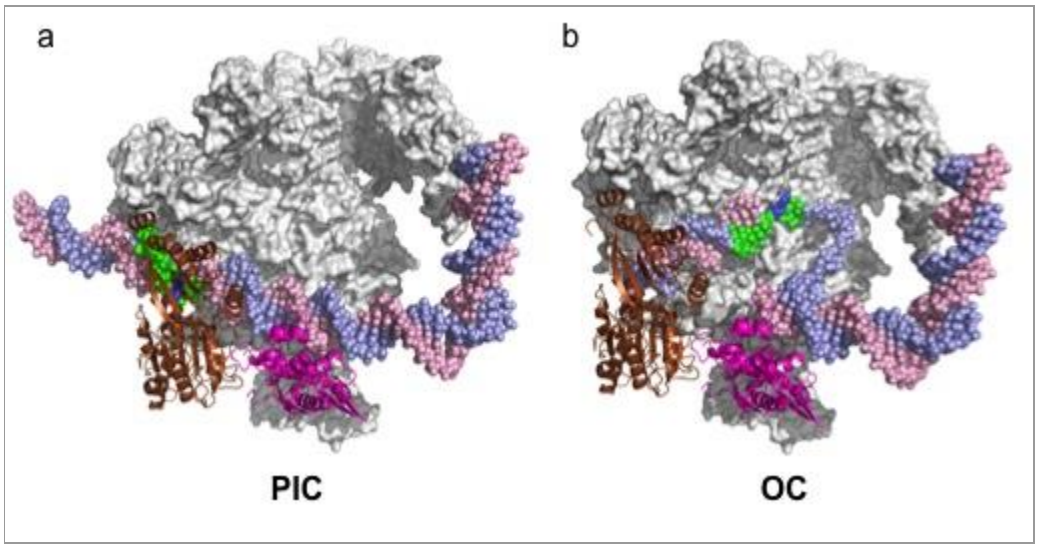

Image courtesy of Dr. Sebastian Grünberg

Updated structural models of the pre-initiation complex (PIC, a) and open complex (OC, b) describe the relative positions of TFIIE (pink) and the Ssl2 subunit of TFIIH (brown) as determined by molecular mapping experiments. In light of these findings, the authors propose that double-stranded DNA sequestered between RNA polymerase II (Pol II, gray) and TFIIE is threaded through the binding site of ATP-bound Ssl2 in the PIC, resulting in the right-handed translocation of the template strand into the Pol II enzyme site, displacement of the non-template strand onto TFIIE, and formation of the OC.